International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 2(C); February 2018; Page No. 9815-9820
DOI: http://dx.doi.org/10.24327/ijcar.2018.9820.1638

Research Article

STABILITY INDICATING ANALYTICAL METHOD VALIDATION FOR DETERMINATION OF RELATED SUBSTANCES BY RPHPLC FOR CYPROTERONE ACETATE & ETHINYL ESTRADIOL IN CYPROTERONE ACETATE & ETHINYL ESTRADIOL TABLET

Asheesh Singh[1]* and Parul Singh[2]

[1]VNS Institute of Pharmacy, Neelbud; Bhopal (MP) – 454775
[2]Department of Pharmacy, Guru Ghasidas University, Bilaspur (India)-495009

A R T I C L E I N F O

Article History:
Received 15th November, 2017
Received in revised form 21st December, 2017
Accepted 23rd January, 2018
Published online 28th February, 2018

Key words:
Cyproterone Acetate, Ethinyl Estradiol, Analytical Method, Validation, High performance Liquid Chromatography.

A B S T R A C T

A simple, accurate, rapid and precise cost effective High performance liquid chromatographic (HPLC) method was validated for the determination of related substances in Cyproterone Acetate and Ethinyl Estradiol in tablet formulation. The high performance liquid chromatography resolution was achieved on symmetry C18 250 x 4.6mm, 5µm, column with an gradient elution at a flow rate of 1.0 mL/min using a mobile phase A as buffer and mobile phase B as acetonitrile. The detection was performed by a photo diode array Detector. The method was validated in the concentration range of Limit of quantitation to 150% of working concentration. The intra and inter-day precision and accuracy were within Limit (10 % Relative Standard Deviation). The overall mean recoveries of Cyproterone Acetate and Ethinyl Estradiol impurities were found within limit.

INTRODUCTION

Cyproterone acetate (CPA) is a steroidal synthetic progestagen and anti-androgenic compound widely administered in patients with metastatic prostate cancer. The first case of CPA-induced fulminant hepatitis with a fatal outcome was reported in 1989. A variety of hepatotoxic reactions have been documented, including immunoallergic cytotoxic reactions, cholestasis, autoimmune hepatitis, acute hepatitis, and fulminant hepatic failure. Despite its low incidence, the prognosis of hepatic failure induced by CPA is fatal. Only 1 of 14 reported patients has survived [1–3]. CPA has been widely prescribed as an anti-androgen to suppress the progression of metastatic prostate cancer. Considering the high use of CPA by urologists late into the treatment process, more discussion about the complication of this drug is needed. It is well-known that patients with prostate cancer have a relatively good prognosis and even patients with bone metastasis can have extended survival periods. Unfortunately, CPA-induced hepatic failure may encroach upon the considerably favourable survival period among patients with metastatic prostate cancer [4-5]. Ethinyl estradiol is also known as ethynyl estradiol (EE) which is a derivative of 17β – estradiol. It is the first orally active semi synthetic steroidal estrogen that is used for the management of menopausal symptoms and female hypogonadism.

Corresponding author:* **Asheesh Singh
VNS Institute of Pharmacy, Neelbud; Bhopal (MP) – 454775

Ethinyl estradiol is an orally bioactive estrogen used in almost all modern formulations of combined oral contraceptive pills. Chemically it is 19-Nor-17α-pregna-1, 3, 5(10)-trien-20-yne-3, 17-diol Interest in pharmaceuticals in the environment has increased substantially in recent years [6-7]. Several studies in particular have assessed human and ecological risks from human pharmaceutical estrogens, such as 17α-ethinyl estradiol (EE2). Regulatory action also has increased, with the USA and other countries developing rules to address estrogens and other pharmaceuticals in the environment [8-9]. Accordingly, the Center for Drug Evaluation and Research at the US Food and Drug Administration has conducted a review and analysis of current data on the long-term ecological exposure and effects of EE2 and other estrogens. The results indicate that mean-flow long-term predicted environmental concentrations (PECs) of EE2 in approximately 99% or more of US surface water segments downstream of wastewater treatment plants are lower than a predicted no-effect concentration (PNEC) for aquatic chronic toxicity of 0.1 µg/L. Exceedances are expected to be primarily in localized, effluent-dominated water segments. The median mean-flow PEC is more than two orders of magnitude lower than this PNEC. Similar results exist for other pharmaceutical estrogens. Data also suggest that the contribution of EE2 more broadly to total estrogenic load in the environment from all sources (including other human pharmaceutical estrogens, endogenous estrogens, natural environmental estrogens, and industrial chemicals), while highly uncertain and variable, appears to be relatively low overall. Additional data and a more comprehensive approach

for data collection and analysis for estrogenic substances in the environment, especially in effluent-dominated water segments in sensitive environments, would more fully characterize the risks [10-15]. A LC-MS/MS method has been reported for simultaneous estimation of Cyproterone Acetate and Ethinyl Estradiol in pharmaceutical dosage forms. No simple and cost effective method has reported so far simultaneous estimation of related substance in Cyproterone Acetate and Ethinyl Estradiol in pharmaceutical dosage forms. Hence the objective of the present study was to develop and validate simple RP-HPLC method for determination of related substances for Cyproterone Acetate and Ethinyl estradiol drug product. This method was validated for specificity, accuracy, precision, linearity and Robustness as per ICH guidelines [16-19].

MATERIAL AND METHOD

Cyproterone Acetate and Ethinyl Estradiol drug product and drug substances information summarized in table no.1.Working standard and Impurity standard used in Experiments reported in table No.2. Apparatus and instruments used in experiment are listed in table No 3. Reagents and solvents used: Water (HPLC grade, Milli Q), Acetonitrile (HPLC grade, JT Baker) Methanol (HPLC grade, JT Baker), Ortho-Phosphoric acid (AR grade).

Preparation of 0.1% OPA Solution: 1 mL of orthophosphoric acid diluted to 1000 mL with milli-Q water and degas.
Mobile Phase A: 0.1% OPA Solution; Mobile Phase B: 100 % Acetonitrile.

Preparation of Diluent: Prepare a degassed mixture of water and acetonitrile in the ratio of 50:50 v/v respectively.

Preparation of standard stock solution–1: Accurately weigh and transfer 30 mg of Ethinylestradiol working standard into a 250 mL volumetric flask, add about 150 mL of Acetonitrile and sonicate to dissolve. Dilute to volume with Acetonitrile and mix well. Transfer 3 mL above solution into a 200 mL of volumetric flask. Dilute to volume with diluent and mix well.
Preparation of standard stock solution–2: Accurately weigh and transfer 30 mg of Cyproterone Acetate working standard into a 50 mL volumetric flask, add about 30 mL of Acetonitrile and sonicate to dissolve. Dilute to volume with Acetonitrile and mix well. Transfer 5 mL above solution into a 50 mL of volumetric flask. Dilute to volume with diluent and mix well.
Preparation of standard solution: Transfer 3 mL of standard stock solution-1 and 5 mL of standard stock solution-2 into a 100 mL of volumetric flask. Dilute to volume with diluent and mix well.

Preparation of placebo solution: Accurately weigh and transfer the placebo about equivalent to 20 mg of Cyproterone Acetate into a 20 mL volumetric flask, add about 15 mL of diluent and sonicate for 30 minutes with occasional shaking, dilute to volume with diluent and mix well. Filter the solution through 0.45 µm PVDF filter. Separately inject 100 µL of diluent (Duplicate), placebo, standard solution (duplicate injections) and sample solution into the chromatographic system. Disregard the peaks due to blank and placebo.

Evaluation of System suitability parameters: The similarity factor between two replicate injections of standard solution should be in between 0.95 to 1.05. The column efficiency as determined from standard solution for Cyproterone Acetate and Ethinylestradiol peaks should not be less than 2000 theoretical plates. The Tailing factor for the same peaks should not be more than 2.0.

Chromatographic conditions:

Column	Symmetry C18, 250 x 4.6 mm, 5.0 µm
Wavelength	210 nm for Ethinylestradiol, 260 nm for Cyproterone Acetate
Flow rate	1.0 mL/min
Injection volume	100 µL
Column Temperature	50°C
Sample Cooler temperature	5°C
Run time	65 mins

Gradient programme:

Time (min)	Mobile phase A	Mobile phase B
0.0	60.0	40.0
15.00	55.0	45.0
35.00	50.0	50.0
45.00	30.0	70.0
55.00	10.0	90.0
60.00	60.0	40.0
65.00	60.0	40.0

RESULT AND DISCUSSION

Specificity: Specificity is the ability of the method to measure the analyte in the presence of process related and the degradation impurities. All known impurity solutions individually, sample solution and spiked sample solution with all known impurities at specification level were prepared and injected into the HPLC equipped with a photodiode array detector and analysed. Peak purity passed for Cyproterone Acetate and Ethinyl Estradiol and their Respective Impurities in control sample and spiked sample. Data is reported in Table No 4 & 5 and Figure No 1 & 2.

Table No 1 Drug Substance and Drug product Information

Name of Drug Product		Cyproterone acetate 2 mg and Ethinylestradiol 0.035 mg Tablets	
Description of Drug Product	:	Light Yellow circular, bi convex film coated tablets. One side debosed as "80" and plain on other.	
Name of Drug Substance	:	Cyproterone acetate	Ethinylestradiol
Description of Drug Substance	:	White or almost white, crystalline powder.	Ethinylestradiol is a white to faintly yellowish white crystalline powder.
Chemical Name	:	6-Chloro-3,20-dioxo-1,2-dihydro-3*H*-cyclopropa [1,2] pregna-1,4,6-trien-17-yl acetate	19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol
Molecular Formula	:	$C_{24}H_{29}ClO_4$	$C_{20}H_{24}O_2$
Molecular Weight	:	416.9	296.40
CAS No.	:	427-51-0	57-63-6

Table No 2 working Standard and Impurity Standard

S No.	Name	Batch No.	Potency (%)
1	Ethinyl Estradiol	AR/WS/008/00	99.0%
2	Cyproterone Acetate	AR/WRS/009/00	99.4%
3	Ethinyl Estradiol Impurity B	SL-017-029	99.49
4	Ethinyl Estradiol Impurity C	SP041M1351V	99.57
5	Ethinyl Estradiol Impurity D	SL-017-026	98.81

Table No 3 List of Instrument Used

S No.	Instrument	Make	Software	Detector/Model No
1	HPLC	Waters	Empower	2489 dual wavelength
2	HPLC	Waters	Empower	2998 PDA Detector
3	Sonicator	Lab India	NA	NA
4	Weight balance	Mettler Toledo	NA	ML204
5	Oven	Thermo lab	NA	GMP
6	Photolytic Chamber	Thermo lab	NA	GMP

Table No 4 Peak purity in standard Solution

Sample	Cyproterone Acetate		Ethinyl Estradiol	
	Purity angle	Purity Threshold	Purity Angle	Purity Threshold
Standard Solution solution	0.266	0.552	10.907	15.338

Table No 5 Peak purity of spiked sample

Sample	Purity angle	Purity Threshold
Cyproterone Acetate	2.469	3.930
Ethinyl Estradiol	14.655	15.675
Impurity C	4.568	6.983
Impurity D	8.969	12.344
Impurity E	17.042	19.284

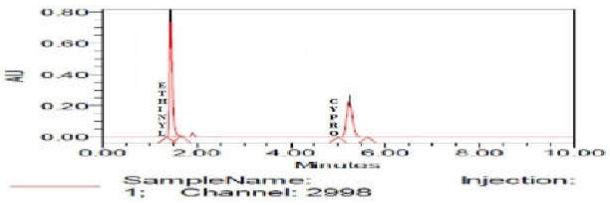

Figure No 1 Standard solution

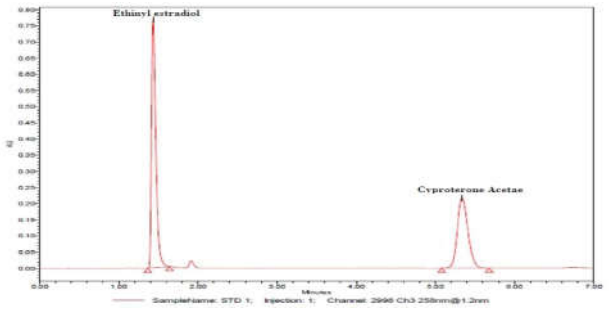

Figure No 2 Control Sample

Limit of Detection (LOD) and Quantitation (LOQ): Limit of Quantitation (LOQ) was established by diluting known concentration of Cyproterone Acetate, Ethinylestradiol and its impurities to achieve the S/N ratio of 10. Six replicates of the LOQ solution was injected in chromatographic system. Limit of Detection (LOD) solution achieved by diluting 3.3 ml of LOQ Solution to 10ml. For Limit of Detection (LOD): The S/N ratio for LOD should be about 3.0. For Limit of Quantitation (LOQ): The S/N ratio for LOD should be about 10.0. % RSD (n=6) of peak area counts of Cyproterone Acetate, Ethinyl Estradiol and its impurity from six replicate injections of predicted LOQ solution should not be more than 10. Based on determination of Prediction linearity, six replicate injections were made for LOD & LOQ. Data is summarized in the given Table No 6.

Linearity: Excellent correlation was achieved for the regression line of Cyproterone Acetate & Ethinyl Estradiol and its related impurities over a range from LOQ to 150 % of the limit level. The correlation coefficient obtained for all the plots was greater than 0.999. The linearity results are tabulated in Table No. 7 & 8.

Table No 6 Limit of Detection and Limit of Quantitation

Limit of Detection		
Name	Area	S/N ratio
Cyproterone Acetate	1827	5.81
Ethinylestradiol	1213	2.23
Ethinylestradiol Related comp B	1094	3.07
Ethinylestradiol Related comp C	1241	2.05
Ethinylestradiol Related comp D	1192	2.83
Limit of Quantitation (S/N ratio)		
Cyproterone Acetate	2518	9.53
Ethinylestradiol	2629	8.96
Ethinylestradiol Related comp B	2673	9.22
Ethinylestradiol Related comp C	2984	12.15
Ethinylestradiol Related comp D	3082	11.59

Table No 7 Table for Linearity of Ethinyl Estradiol and Impurity C

Level	Concentration (µg/ml)	Ethinyl Estradiol	Concentration (µg/ml)	Impurity C
LOQ	0.040	3098	0.010	1845
Lin-1	0.153	11692	0.119	20921
Lin-2	0.382	29836	0.297	52779
Lin-3	0.611	47481	0.475	84098
Lin-4	0.763	59031	0.593	104876
Lin-5	0.916	70977	0.712	125812
Lin-6	1.145	88446	0.890	157783
	Slope	77337	Slope	177044
	Intercept	63	Intercept	-2
	Correlation Coefficient	0.99999	Correlation Coefficient	1.00000

Table No 8 Table for Linearity of Impurity B and Impurity D

Level	Concentration (µg/ml)	Impurity B	Concentration(µg/ml)	Impurity E
LOQ	0.054	3718	0.025	3512
Lin-1	0.083	6035	0.080	11530
Lin-2	0.207	15403	0.201	28680
Lin-3	0.331	24133	0.322	45424
Lin-4	0.414	30207	0.402	56421
Lin-5	0.496	36131	0.482	67689
Lin-6	0.620	45117	0.603	84106
	Slope	72900	Slope	139380
	Intercept	0	Intercept	368
	Correlation Coefficient	0.99995	Correlation Coefficient	0.99997

Accuracy: The studies were carried out at four different levels: LOQ, 50%, 100%, and 150% of limits. The percentage of recoveries of Imp-B, Imp-C and Imp-D were calculated with respect to amount spiked and amount recovered. The percentage recovery at each level was calculated against the Cyproterone acetate and Ethinyl Estradiol standard. Mean recovery should be in the range of 90.0% to 110.0% for 50%, 100% and 150% levels and 85% to 115% for LOQ level. Mean recovery in percentage is reported in Table No. 9.

Table No 9 Accuracy of Impurity of Ethinylestradiol

	Mean Recovery (%)			
Name of Impurity	LOQ	50	100	150
Impurity C	92.5	98.2	99.1	104.5
Impurity D	94.6	103.2	102.3	101.2
Impurity E	98.2	101.3	100.7	99.9

Precision: Precision is the closeness of agreement between a series of measurements obtained from multiple sampling of same sample under the prescribed conditions. Quantification of

individual impurities and Cyproterone Acetate and Ethinylestradiol Tablets was performed for each of the preparations and the percent relative standard deviation (RSD) was determined for the content of the impurities. To evaluate the intermediate precision, the same experiment was repeated with a different lot of column and a different instrument in the same laboratory. Precision data reported in table No.10, 11& 12.

Table No 10 Over all %RSD Comparison for Impurities in Precision and Ruggedness study

Test	Ethinylestradiol Impurity B (%w/w)		Ethinylestradiol Impurity C (%w/w)	
	Method Precision	Ruggedness	Method Precision	Ruggedness
1	0.078	0.078	Below LOQ	Below LOQ
2	0.077	0.079	Below LOQ	Below LOQ
3	0.078	0.076	Below LOQ	Below LOQ
4	0.080	0.079	Below LOQ	Below LOQ
5	0.076	0.076	Below LOQ	Below LOQ
6	0.079	0.076	Below LOQ	Below LOQ
Mean	0.078		NA	
SD	0.0014		NA	
% RSD	1.79		NA	

Table No 11 Over all %RSD Comparison for Impurities in Precision and Ruggedness study

Test	Ethinylestradiol Impurity D (%w/w)		Ethinylestradiol Total Impurity (%w/w)	
	Method Precision	Ruggedness	Method Precision	Ruggedness
1	Below LOQ	Below LOQ	0.078	0.078
2	Below LOQ	Below LOQ	0.077	0.079
3	Below LOQ	Below LOQ	0.078	0.076
4	Below LOQ	Below LOQ	0.080	0.079
5	Below LOQ	Below LOQ	0.076	0.076
6	Below LOQ	Below LOQ	0.079	0.076
Mean	NA		0.078	
SD	NA		0.0014	
% RSD	NA		1.79	

Table No. 12 Over all %RSD Comparison for Impurities in Precision and Ruggedness study

Test	Cyproterone acetate single maximum Impurity (%w/w)		Cyproterone acetate Total Impurity (%w/w)	
	Method Precision	Ruggedness	Method Precision	Ruggedness
1	BLQ	BLQ	BLQ	BLQ
2	BLQ	BLQ	BLQ	BLQ
3	BLQ	BLQ	BLQ	BLQ
4	BLQ	BLQ	BLQ	BLQ
5	BLQ	BLQ	BLQ	BLQ
6	BLQ	BLQ	BLQ	BLQ
Mean	NA		NA	
SD	NA		NA	
% RSD	NA		NA	

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. Deliberate changes were made from original experimental conditions to record the tailing factor and theoretical plates of the Cyproterone Acetate and Ethinylestradiol Tablets to determine the robustness of the developed method. Data reported in Table No.13.

Stability of Analytical solution: The solution stability of sample and standard solution provide an indication of the method's reliability in normal usage during the storage of the solutions used in the method. No significant changes were experienced in the content of any of the impurities during solution stability. The % Cumulative RSD of Standard solution and sample Solution Reported in Table No.14 & 15.

Table No 13 Robustness, RRT

Sr. no.	Parameters	Variations	RRT of Related Compounds		
			Impurity-B	Impurity-C	Impurity-D
1	Control-1	-	0.16	0.51	0.75
	Control-2		0.16	0.51	0.74
2	Column Temperature	-5°C	0.16	0.51	0.75
		+5°C	0.16	0.51	0.75
3	Flow rate	-0.1ml/min	0.17	0.52	0.76
		+0.1ml/min	0.15	0.50	0.75
4	Wavelength	-5 nm	0.16	0.51	0.74
		+5 nm	0.16	0.51	0.74

Table No 14 Table for solution stability for diluted standard at room temperature

Sr. No.	Time (hrs)	(Area) Cyproterone Acetate	(Area) Ethinylestradiol
1	Initial	109489	48385
2	31	109901	48735
3	43	109900	48208
4	56	109934	48725
5	64	109708	48519
6	75	109758	48472
7	86	110127	49678
8	96	109238	49778
9	107.00	109863	49935
Cumulative % RSD		0.24	1.36

Table No 15 Table for solution stability for sample solution preparation at Room Temperature

Sr. No.	Time (hrs)	Ethinylestradiol	Cypro Acetate	Area Imp C	Area Imp D	Area Imp E
1	Initial	326493	23063582	13060	4225	34388
2	40.00	325194	22881482	12974	4191	35267
3	51.00	322697	19929588	12948	4212	35299
4	61.00	327863	19005600	12932	4263	35304
Cumulative % RSD		0.68	9.71	0.44	0.72	1.29

Table No 16 System Suitability for Cyproterone acetate

Validation parameter	Column Efficiency	Tailing Factor	%RSD	Similarity Factor
Specificity	36159	1.03	0.46	1.00
Method precision/Soln Stab	41799	1.06	0.14	1.00
Ruggedness	39266	1.07	0.09	0.99
Limit of Quantitation	35905	1.05	0.12	1.00
Limit of Detection	33340	1.04	0.25	1.00
Accuracy	39456	1.03	0.98	0.99
Linearity	38564	1.04	0.43	0.99
Robustness	32987	1.06	0.34	1.0

Table no 17 System Suitability for Ethinylestradiol

Validation parameter	Column Efficiency	Tailing Factor	%RSD	Similarity Factor
Specificity	15685	0.91	1.47	1.02
Method precision/Soln Stab	14815	1.09	1.04	1.01
Ruggedness	14412	1.08	0.70	1.01
LOQ	14002	1.10	0.67	1.00
LOD	13832	1.10	0.46	0.99
Accuracy	14245	1.10	0.99	1.02
Linearity	14056	1.09	0.87	1.01
Robustness	13732	1.03	0.65	0.99

System Suitability: The similarity Factor for Cyproterone acetate and Ethinylestradiol from two replicate injections of diluted standard solution should be in between 0.95 to 1.05.

The column efficiency determined for the Cyproterone acetate and Ethinylestradiol from standard solution should not be less than 2000 theoretical plates and the tailing factor for the same peaks should not be more than 2.0. The % RSD of area for six replicate injections of standard solution of Cyproterone acetate and Ethinylestradiol should not be more than 2.0. System Suitability reported in table No 16 & 17 respectively.

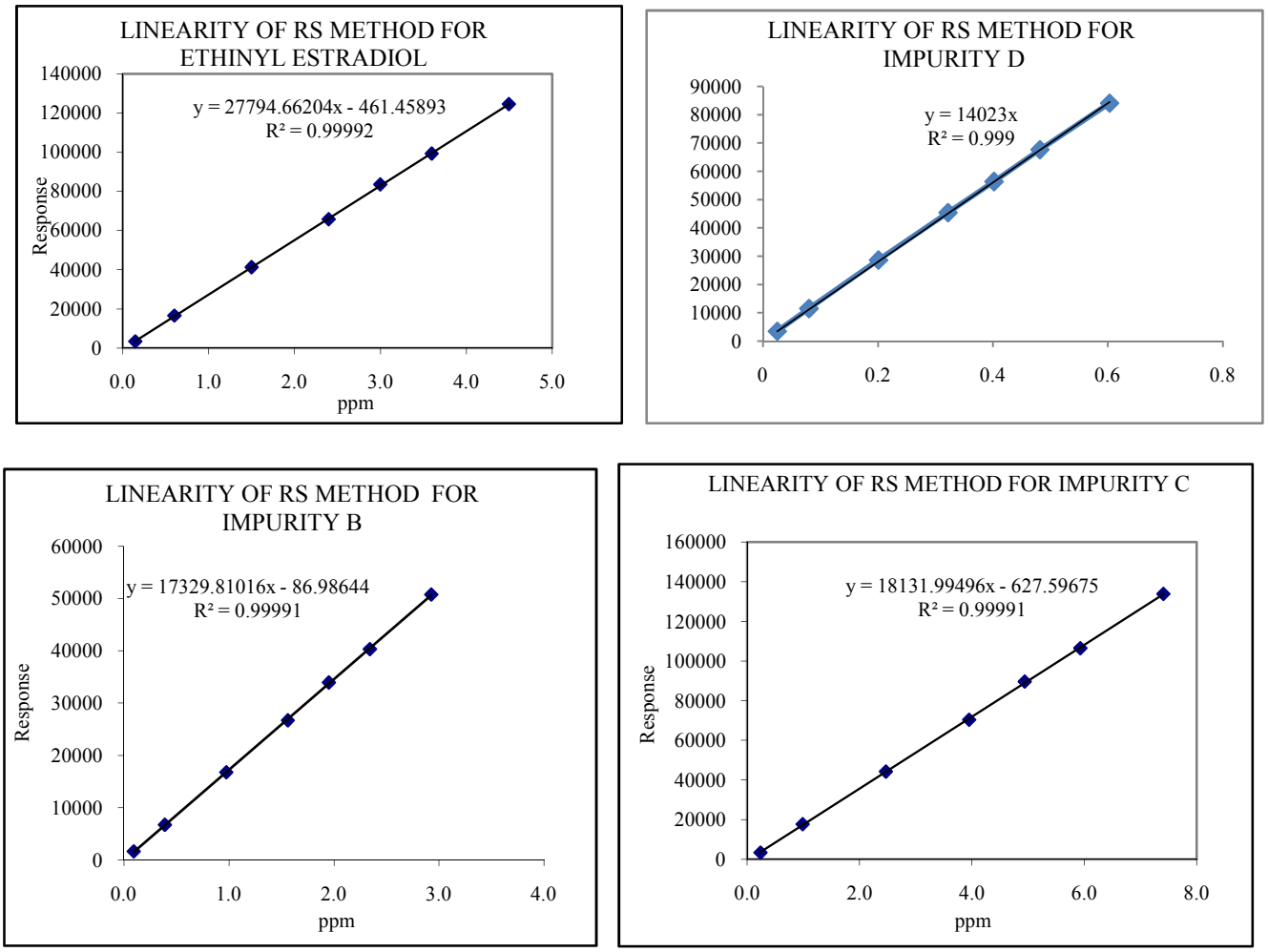

Figure No.3 Linearity graph of Ethinyl Estradiol & Impurity B, C & D

SUMMARY AND CONCLUSION

The Validated HPLC method for related substance of Cyproterone Acetate and Ethinylestradiol Tablets is linear, precise, accurate and specific. The results of the validation carried out for the method satisfied the ICH requirements. This method can be used for the detection and quantification of known, unknown and degradation impurities in the Cyproterone Acetate and Ethinylestradiol Tablets during routine analysis and also for stability studies in view of its capability to separate degradation products.

Acknowledgements

The authors wish to thank the management of Alpa Laboratories Limited Pithampur for supporting this work. Authors wish to acknowledge the Analytical research group for providing the necessary facilities for our research and also wish to thanks colleagues in Validation division of analytical research for their co-operation in carrying out this work.

List Ofabbreviations

No.	Number
NA	Not Applicable
BLQ	Below limit of Quantification
LOQ	Limit of Quantitation
SD	Standard Deviation
RSD	Relative Standard Deviation
LOD	Limit of Detection
Imp	Impurity
Unk	Unknown
Max	Maximum
Hrs	Hours
HPLC	High performance Liquid Chromatography
RSD	Relative Standard Deviation
RRT	Relative retention time
S/N	Signal to Ratio

References

1. Savidou I, Deutsch M, Soultati AS. Hepatotoxicity induced by cyproterone acetate: a report of three cases. *World J Gastroenterol*. 2006; 12:7551–7555.

2. Levesque H, Trivalle C, Manchon ND. Fulminant hepatitis due to cyproterone acetate. *Lancet*. 1989; 1:215–226.
3. Murphy BJ, Collins BJ. Severe hepatitis and liver failure induced by cyproterone acetate. *Aust N Z J Med*. 1996; 26:724-731.
4. Kacar S, Akdogan M, Kosar Y. Estrogen and cyproterone acetate combination induced autoimmune hepatitis. *J Clin Gastroenterol*. 2002; 35:98–100.
5. Drakos PE, Gez E, Catane R. Hepatitis due to cyproterone acetate. Eur J Cancer. 1992; 28:1931–1932.
6. Pradad G, Babu PS, Ramana MV. Validated RP-HPLC method for the estimation of drospirenone in formulation. *Int J of Research Pharm and Bio Sci*. 2011; 2:1341-1345.
7. Sweetman SC. Drospirenone. In editor. Martindale: The complete drug reference. *London Pharmaceutical* Press: 2009; 5: 2095-2099.
8. Birtle A. Maximizing survival in metastatic castrate-resistant prostate cancer: A clinical viewpoint. *Expert Rev Anticancer Ther*. 2013; 13:89–99.
9. Daughton CG. Published literature relevant to the issues surrounding PPCPs as environmental contaminants. In: EPA US.2012.
10. Brooks BW, Berninger JP, Kristofco LA, Ramirez AJ, Stanley JK, Valenti TW. Pharmaceuticals in the environment: lessons learned for reducing uncertainties in environmental risk assessment. *Prog Mol Biol Transcl Sci.* 2012; 112:231–258.
11. UNEP. State of the science of endocrine disrupting chemicals examined in landmark UN report. UNEP News Centre. 2013.
12. USFDA. National Environmental Policy Act; Revision of Policies and Procedures; final rule. Federal Register; 1997.
13. Mostafa AA, Refaat HH. Different methods for the determination of gestodene, and cyproterone acetate in raw material and dosage forms. *Journal of Pharma and Bio. Analy.* 2001; 25:425-436.
14. Wise A, O'Brien K, Woodruff T. Are oral contraceptives a significant contributor to the estrogenicity of drinking water? *Environ Sci Technol*. 2011; 45:51–60.
15. Anderson PD, Johnson AC, Pfeiffer D, Caldwell DJ, Hannah R, Mastrocco F. Endocrine disruption due to estrogens derived from humans predicted to be low in the majority of U.S. surface waters. *Environ Toxicol Chem*. 2012; 31:1407–1415.
16. FDA, Food and Drug Administration. Center for Drug Evaluation and Research (CDER), Guidance for Industry "Bioanalytical Methods Validation for Human Studies". U.S. Department of Health and Human Services; 2001.
17. International Conference on Harmonization Q1A (R2)) Stability Testing of New Drug Substances and Products. 29. International Conference on Harmonization Q3A (R2) Impurities in New Drug Substances.
18. Pavic K, Cudina O, Agbaba D, Vladimirov S. Quantitative analysis of cyproterone acetate and ethinylestradiol in tablets by use of planar chromatography. *Journal of Planar Chrom*. 2003; 16: 45-47.
19. Souri E, Jalalizadeh H, Farsam H, Ghadiri R. Simultaneous determination of cyproterone acetate and ethinylestradiol in tablets by derivative spectrophotometry. *Chem Pharm Bull*. 2005; 53:949-951.

How to cite this article:

Asheesh Singh and Parul Singh (2018) 'Stability Indicating Analytical Method Validation For Determination of Related Substances by Rphplc for Cyproterone Acetate & Ethinyl Estradiol in Cyproterone Acetate & Ethinyl Estradiol Tablet', *International Journal of Current Advanced Research,* 07(2), pp. 9815-9820.

****